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(54) **A METHOD FOR MASS PRODUCTION OF TAXOL FROM TAXUS GENUS PLANT**

MASSENHERSTELLUNGSVERFAHREN VON TAXOL AUS PFLANZEN DER GATTUNG TAXUS

PROCEDE DE PRODUCTION EN MASSE DE TAXOL A PARTIR D'UNE PLANTE DU GENRE TAXUS

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WO-A-92/18492
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Description

BACKGROUND OF THE INVENTION

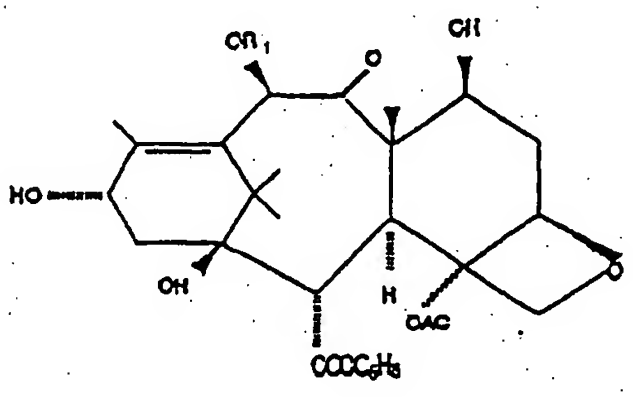
Field of the Invention

[0001] The present invention relates to a method for mass production of taxol from Taxus genus plant, more specifically, to a rapid and simple method for mass production of taxol with a high purity and recovery, which comprises the steps of solvent extraction of biomass from Taxus genus plant employing methanol, dichloromethane and hexane, adsorbent treatment, precipitation in hexane, fractional precipitation and high performance liquid chromatography.

Description of the Prior Art

[0002] Taxanes are diterpene compounds containing the taxane skeleton. For example, taxol is famous as the first identified compound with a taxane ring which is effective for the treatment of leukemia and cancer. Recently, it has been reported that taxol is capable of curing approximately 30%, 50% and 20% of ovarian, breast and lung cancer patients, respectively. Also, taxane compounds include baccatin III, 10-deacetylbaccatin III, 10-deacetyltaxol, cephalomannine and deacetylcephalomannine, which are employed in the semi-synthesis of taxol.

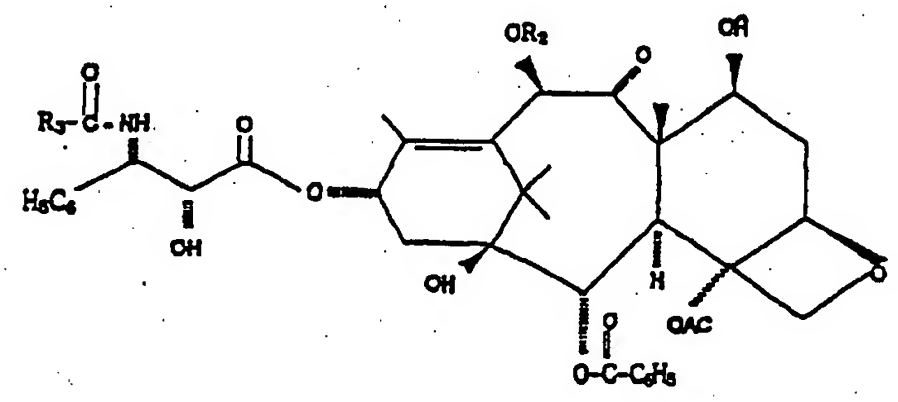
[0003] Taxane compounds are represented as general formula (I) and (II) as followings:



(I)

wherein,

if R_1 = AC, the compound is baccatin III; and,
if R_1 = OH, the compound is 10-deacetylbaccatin III.



(II)

wherein,

if R_2 = AC, R_3 = C_6H_5, the compound is taxol;
if R_2 = OH, R_3 = C_6H_5, the compound is 10-deacetyltaxol;
if R_2 = AC, R_3 = $CH_3CH=CH(CH_3)$, the compound is cephalomannine; and,
if R_2 = OH, R_3 = $CH_3CH=CH(CH_3)$, the compound is 10-deacetylcephalomannine.

[0004] On the other hand, total synthesis, semi-synthesis and extraction methods have been employed to prepare taxol.

[0005] The total synthesis method, however, has not been practically applied in the art, since it requires very expensive chemical reagents and the yield is not so high, which can be expected from the complicated chemical structure of taxol.

[0006] The semi-synthesis method employing precursors such as 10-deacetylbaccatin III, has revealed some drawbacks since it essentially entails complicated and multiple steps of isolating and purifying taxol precursors from Taxus genus plant and transforming the taxol precursors to taxol, which, in turn, has been an obstacle to the universal use of the method.

[0007] Accordingly, extraction methods by which taxol can be isolated from Taxus genus plants in a direct manner, have prevailed in the art, since they have the advantage of economy, and a variety of approaches have been described in the art:

[0008] WO 92/18492 describes a method of extracting and purifying taxol and other taxanes from taxane-containing plants. According to this document four process steps (A to D) are performed for obtaining a so-called second residue, which contains taxol/taxanes. Said second taxol-containing residue is subjected to further finishing treatment steps (E and F, respectively). Step E is directed to the adsorption of the ingredients of the second residue onto a solid support like celite. Subsequently the taxanes forming a coating on the solid support are sequentially eluted, i.e. desorbed from the solid support. Alternatively in step F the second residue is partitioned between an aqueous phase of water and a polar organic solvent miscible with water.

[0009] US-A 5,279,949 describes a process for the separation of taxanes and taxol. For extracting the starting plant material a mixture of about 50% to 80% ethanol in water is used. After decolorizing the crude taxane mixture with charcoal taxanes and taxol are chromatographically separated.

[0010] WO 93/17121 describes a process for the enhanced production and recovery of taxol and taxanes by cell cultures of Taxus species. Taxol and/or taxanes are extracted by using methanol or a mixture of methylene chloride and isopropyl alcohol.

[0011] WO 94/12268 discloses a method of isolating taxol by employing a semi-permeable membrane and reverse osmosis apparatus. However, said method has revealed a serious problem that it essentially requires the expensive semi-permeable membrane and reverse osmosis apparatus accompanied by complicated techniques for operating them.

[0012] EP 553,780 A teaches a method of isolating taxol and precursor thereofs which comprises the steps of vacuum drying a methanol extract of Taxus genus plant, solvent extraction employing cyclohexane and methylenechloride to give crude taxol followed by silica gel column HPLC.

[0013] WO 92/07842 illustrates a method isolating taxol Which comprises the steps of a series of solvent extractions employing ethanol, chloroform and methanol to give crude taxol followed by reverse-phase HPLC.

[0014] WO 94/13827 suggests a method of purifying taxol which comprises the steps of organic solvent extraction using ethanol, methanol and acetone, adsorbent treatment of activated carbon or charcoal to give crude taxol followed by normal-phase liquid chromatography.

[0015] JP 6-157329 A offers a method of obtaining crude taxol of low-purity which comprises a series of solvent extractions employing ethylacetate, ether, acetonitrile and acetone.

[0016] However, all of the prior art purification methods obtain crude taxol of low-purity by employing solvent extraction and chromatography works, wherein the taxol of interest is accompanied by taxol-related compounds such as terpenoids, lipids, chlorophyll and phenols. Accordingly, high-purity of taxol has not been obtained even in the case of employing so many chromatographic columns, leading to a heavy load of impurities on the columns used in the purification steps.

[0017] Moreover, since the purity of taxol thus obtained is not high, solubility in organic solvent is naturally so low that recovery and yield in the course of chromatography can not be controlled and extra steps for crystallization are essentially required to obtain high-purity crystallized taxol. Accordingly, the prior art purification methods have not been practically employed in the art and there has been a continuous need in the art to develop a method for isolating high-purity taxol in a more simple and economical manner.

SUMMARY OF THE INVENTION

[0018] In accordance with the present invention, the inventors developed a method of purifying taxol from Taxus genus plant by employing a series of solvent extractions, adsorbent treatment, fractional precipitation and high performance liquid chromatography.

[0019] A primary object of the present invention is, therefore, to provide a rapid and simple method for mass production of taxol from Taxus genus plant with a high purity of over 99% and a high recovery of over 90% by employing small amounts of organic solvent, regardless of the water content in biomass.

DETAILED DESCRIPTION OF THE INVENTION

[0020] The method for mass production of taxol from Taxus genus plant of the present invention, comprises the steps of:

(i) organic solvent extraction of biomass from Taxus genus plant in a mixture of dichloromethane/methanol or methanol, stirring and filtering to obtain a crude extract,

(ii) synthetic adsorbent treatment of said crude extract at a ratio of 10 to 200% (w/w), stirring and filtering to obtain a filtrate,

(iii) addition of 500 to 1500% volume of hexane to the filtrate in dichloromethane to precipitate crude taxol,

(iv) fractional precipitation of the crude taxol in a mixture of methanol and water at a ratio of 1 to 10% (w/v) and drying the precipitate to obtain taxol powder, and

(v) high performance liquid chromatography (HPLC) of said taxol powder.

[0021] The method is advantageously developed by the measures mentioned in the dependent claims.

[0022] Biomass employed in the present invention as starting material includes: the leaf or bark of Taxus genus plant which is chopped and powdered and the cake of cell mass obtained in tissue culture of Taxus genus plant, where Taxus genus plant covers Taxus brevifolia, Taxus canadensis, Taxus cuspidata, Taxus baccata, Taxus globosa, Taxus floridana, Taxus wallichiana, Taxus media and Taxus chinensis, regardless of the water content in the biomass.

[0023] The method for mass production of taxol of the invention is described in more detail, in accordance with the purification steps.

Step 1: Organic solvent extraction

[0024] As a preferred embodiment of the present invention, the solvent extration is carried out employing methanol and dichloromethane as follows:

[0025] The biomass of Taxus genus plant is added to methanol, stirred at room temperature for 20 to 60min, preferably 30 to 40 min and filtered to give a methanol extract, where the biomass is preferably added to methanol at a ratio of 20 to 200%(w/v), preferably 40 to 140%(w/v), most preferably 100%(w/v), and extraction is repeated at least 3 times, preferably 4 times. Then, the methanol extracts obtained from each time, are collected and concentrated at a temperature of 20 to 40°C under a reduced pressure of 1 to 30mmHg, to reduce the volume of the methanol extract to 20 to 30% of original. At this time, careful attention should be drawn to control the temperature in a range of 20 to 40°C, since epimerization of taxol and taxol derivatives may be accelerated at a temperature of over 40°C. To the concentrated methanol extract is added dichloromethane at a volume ratio of 10 to 50%, preferably 20 to 30%, stirred at room temperature for 10 to 20min, and left to stand to obtain a crude extract. Extraction is repeated at least 2 times, preferably 3 times and the crude extracts thus obtained are pooled and dried at 20 to 40°C under a reduced pressure of 1 to 30mmHg.

[0026] Alternatively, the solvent extraction can be carried out by employing dichloromethane/methanol, methanol and hexane as followings:

[0027] The biomass of Taxus genus plant is added to a mixture of dichloromethane/methanol, stirred at room temperature for 20 to 60min, preferably 30 to 40 min and filtered to give a dichloromethane/methanol extract. At this moment, dichloromethane and methanol are preferably mixed at a volume ratio of 7:3 to 9:1, preferably 8:2 to 9:1, most preferably 9:1, and the biomass is added to the mixture at a ratio of 10 to 100%(w/v), preferably 15 to 50%(w/v). Then, the extraction and concentration are carried out analogously as in the solvent extraction illustrated above. The concentrated extract is dissolved in methanol at a ratio of 50 to 200%(w/v), preferably 70 to 150%(w/v), more preferably 90 to 110%(w/v), to obtain methanol extract. The methanol extract is added to hexane at a volume ratio of 5 to 30%, preferably 7 to 20%, more preferably 8 to 15%, stirred at room temperature for 10 to 20min and left to stand, and followed by the removal of hexane layer to obtain a crude extract.

Step 2: Adsorbent treatment

[0028] Since the impurities such as tar in the dried crude extract obtained in step 1, play an obstructive role in a subsequent purification step, synthetic adsorbent is added to remove the impurities. To the crude extract is dissolved in dichloromethane at a ratio of 5 to 100%(w/v), preferably 10 to 50%(w/v), more preferably 15 to 25%(w/v) and followed

by the addition of the synthetic adsorbent at a ratio of 10 to 200%(w/w), preferably 30 to 100%(w/w), most preferably 50%(w/w), stirred at 30 to 40°C for 10 to 40 min and filtered with the said synthetic adsorbent to obtain filtrate. The synthetic adsorbents used are active clay, activated charcoal and activated carbon, etc., from which active clay is most preferably used. The filtrate thus obtained is washed with a proper amount of dichloromethane several times and washings are combined with the filtrate. Then, the filtrate thus combined is concentrated at 30 to 40°C under a reduced pressure of 1 to 30mmHg to the level equivalent to 150 to 200% of the crude extract prior to adsorbent treatment.

Step 3: Precipitation in hexane

[0029] The filtrate obtained in step 2 is added to 500 to 1,500% volume of hexane, preferably 700 to 1,200%, most preferably 1,000% to obtain the precipitate, and filtrated to give crude taxol whose taxol content is over 23%.

Step 4: Fractional precipitation

[0030] The crude taxol obtained in the previous step, is dissolved in a mixture of alcohol and distilled water at a ratio of 1 to 10%(w/v) and left to stand at -20 to 10°C for 1 to 3 days to obtain taxol precipitate. Then, the resultant precipitate is filtered and dried at 20 to 40°C for 1 to 3 hrs under a vacuum condition, to give taxol powder. At this moment, methanol and distilled water are preferably mixed at a volume ratio of 2:1 to 1:1. The fractional precipitation is repeated several times, preferably at least 2 times to obtain taxol of over 85% purity, which also guarantees high recovery and purity while minimizing the load borne on the columns employed in a subsequent HPLC step.

Step 5: High performance liquid chromatography (HPLC)

[0031] HPLC step according to the embodiment of claim 9 is composed of an HPLC employing a hydrophobic resin column, e.g., ODS(octadecylsilylated, C_{18}) column to remove non-polar impurities, and an HPLC employing a silica column to remove polar impurities.

[0032] In an HPLC employing a hydrophobic resin column, taxol powder dissolved in organic solvent is loaded on the hydrophobic resin column, e.g., ODS, and elution is made with a mixture of methanol and water. Then, the eluates are analyzed by UV detector by determining absorbances at 227nm and active fractions containing taxol are pooled, and dried under a vacuum condition for a subsequent use in silica HPLC. At this time, taxol powder is dissolved in dimethylsulfoxide(DMSO) or methanol at a ratio of 0.5 to 10%(w/v), preferably 1 to 2%(w/v) and methanol and water are mixed at a volume ratio of 1:0.3 to 1:0.8, preferably 1:0.4 to 1:0.7, more preferably 1:0.5 to 1:0.6. Samples are injected onto the HPLC at a speed of 3 to 5cm/min at a concentration of 50 to 150mg/ml(in methanol).

[0033] In an HPLC employing a silica column, taxol containing fractions obtained in the previous HPLC work, are injected onto the silica column and eluted with a mixture of dichloromethane and methanol. Then, the eluates are analyzed by a UV detector by determining absorbances at 227nm and active fractions are pooled, and dried under a vacuum condition to give the crystallized taxol. At this time, samples are injected onto the HPLC at a concentration of 50 to 150mg/ml(in CH_2Cl_2), and eluted with a mixture of dichloromethane and methanol mixed at a volume ratio of 95:5 to 99:1, preferably 98:2 to 99:1, most preferably 99:1. The HPLC steps finally produce taxol crystals of over 99% purity with a recovery of over 90%.

Quantitative Analysis of Taxol

[0034] Taxol which is purified from Taxus genus plant according to the method of the present invention, is quantitatively assayed by employing high performance liquid chromatography under a specific condition described in Table 1 below.

Table 1:

Condition for quantitative assay of taxol	
Instrument	HPLC(Waters, U.S.A.)
Column	Capcell Pack C_{18} UG 120 (length: 250mm, inner diameter: 4.6mm)
Column temp.	40°C
Mobile phase	CH_3CN : water(20 - 100% gradient)
Fluid speed	1.0 ml/min

Table 1: (continued)

Condition for quantitative assay of taxol	
Injection volume	10 µl
Detector	UV(227nm), ATTE = 3

[0035] The present invention is further illustrated in the following examples, which should not be taken to limit the scope of the invention.

Example 1: Purification of taxol from the tissue culture of the Taxus genus plant(I)

[0036] To 8kg of biomass obtained from the tissue culture of Taxus genus plant was added 8L of 95%(v/v) methanol, stirred at room temperature for 30min, and filtered to give a methanol extract. Extraction was repeated 3 times, and the methanol extracts obtained from each time were pooled and concentrated at 35°C under a reduced pressure of 30mmHg, to give 4L of methanol extract concentrate. Purity of taxol in the methanol extract was 0.24% and recovery was 100%. To the concentrated methanol extract was added 1.5L of dichloromethane, stirred at room temperature for 15min, and left to stand to obtain dichloromethane extract, and the extraction was repeated 3 times. Purity of taxol the crude extract was 2.4% and recovery was 100%.

[0037] The crude extract was dried at 35°C under a pressure of 30mmHg, and 15g of the extract was dissolved in 56ml of dichloromethane. Then, to the resultant solution was added 35g of the synthetic adsorbent of active clay, stirred at 40°C for 20min and filtered to obtain filtrate. The filtrate thus obtained was washed with 285ml of dichloromethane 3 times and washings were combined with the filtrate. Then, . the filtrate thus combined was concentrated at 35°C under a reduced pressure of 20mmHg, to obtain 100ml of final concentrate. Purity of the extract was 3.1% and recovery was 97%. 100ml of dichloromethane extract was added to 1L of hexane to obtain the precipitate, and filtered to give crude taxol of 23% purity(recovery 95%).

[0038] 1g of crude taxol thus obtained was dissolved in 28.75ml of methanol, and to the solution was added 17.25ml of distilled water and left to stand at 4°C for 2 days to obtain crystallized taxol. Then, the resultant solution was filtered with a 0.4µm filter and dried at 35°C for 2 hrs under a vacuum condition, to give 299mg of taxol powder of 70% purity (recovery 91%).

[0039] 299mg of taxol powder dissolved in 3ml of methanol was injected to an ODS C_{18} column(ϕ50×500mm), and eluted with a mixture of methanol and water of 65:35(v/v) under an assay condition summarized in Table 2 below. Then, the eluates were analyzed by UV detector by determining absorbances at 227nm and active fractions having retention time(Rt) of 40 to 70min were collected. Purity of taxol was 90% and recovery was 90%. Taxol containing fractions were dried at 35°C under a reduced pressure of 10mmHg. 209.3mg of sample was dissolved in 2ml of dichloromethane and injected to a silica column and eluted with a mixture of dichloromethane and methanol of 100:1.2(v/v) under an assay condition summarized in Table 3 below. Then, the eluates were analyzed by UV detector and active fractions containing taxol were collected, dried by rotary evaporator, and vacuum dried to give 170.5mg of crystallized taxol(purity 99.5%).

Table 2:

Assay condition for HPLC employing ODS column	
Instrument	Waters Delta Prep 4000 HPLC(Waters, U.S.A.)
Column	ODS C_{18} column (ϕ50mm×500mm, Shiseido, Japan)
Fluid speed	80ml/min
Injection volume	299mg/3ml(in methanol)
Detector	UV(227nm)

Table 3:

Assay condition for HPLC employing silica column	
Instrument	Waters Delta Prep 4000 HPLC(Waters, U.S.A.)
Column	Silica column (ϕ50mm×500mm, Shiseido, Japan)
Fluid speed	80ml/min

Table 3: (continued)

Assay condition for HPLC employing silica column	
Injection volume	209.3mg/2ml(in CH_2Cl_2)
Detector	UV(227nm)

Example 2: Purification of taxol from the tissue culture of the Taxus genus plant(II)

[0040] Taxol was purified from the tissue culture of the Taxus genus plant analogously as in Example 1, with the exception of solvent extraction employing dichloromethane/methanol, methanol and hexane: To 10kg of biomass of Taxus genus plant was added 45L of dichloromethane/methanol mixture(9:1, v/v), stirred at room temperature for 20 to 60min, preferably 30 to 40 min and filtered to give a dichloromethane/methanol extract. Then, the extraction and concentration were carried out analogously as in the solvent extraction of Example 1. 100g of the concentrated extract was dissolved in 100ml of methanol, to obtain a methanol extract. Purity of taxol in the methanol extract was 0.59% and recovery was 95%. 100ml of the methanol extract was added to 1L of hexane, stirred at room temperature for 15min and left to stand, and followed by the removal of hexane layer 3 times, to obtain a crude extract. Purity of taxol in the crude extract was 2.8% and recovery was 100%.

[0041] HPLC analysis of taxol finally obtained revealed 99.5% purity and 90% recovery.

Example 3: Purification of taxol from the leaf or bark of Taxus genus plant(I)

[0042] Taxol crystal was purified analogously as in Example 1, with the exception of employing the chopped and powdered leaf or bark of Taxus genus plant as starting material. HPLC analysis of taxol finally obtained revealed 99.6% purity and 96% recovery.

Example 4: Purification of taxol from the leaf or bark of Taxus genus plant(II)

[0043] Taxol crystal was purified analogously as in Example 2, with the exception of employing the chopped and powdered leaf or bark of Taxus genus plant as starting material. HPLC analysis of taxol finally obtained revealed 99.6% purity and 90.5% recovery.

Examples 5 to 6:

[0044] Taxol crystal was purified analogously as in Examples 1 and 3, with the exception of using 32L of methanol and lengthening stirring time to 40min in the methanol extraction step. HPLC analysis revealed 99.6% purity.

Examples 7 to 8:

[0045] Taxol crystal was purified analogously as in Examples 1 and 3, with the exception of using 4L of dichloromethane and lengthening stirring time to 20min in the methanol/dichloromethane extraction step. HPLC analysis revealed 99.5% and 96% purity, respectively.

Examples 9 to 10:

[0046] Taxol crystal was purified analogously as in Examples 1 and 3, with the exception of employing methanol instead of dimethylsulfoxide as a solvent for dissolution of sample in the HPLC step. HPLC analysis revealed 99% and 97% purity, and 91%, 92% recovery, respectively.

Comparative Example:

[0047] Taxol powder was obtained analogously as in Example 1, with the exception of skipping the active clay treatment and fractional precipitation steps. HPLC analysis revealed about 40% purity.

[0048] As clearly illustrated and demonstrated above, the present invention provides a method for mass production of taxol from Taxus genus plant with a high purity of over 99% and a high recovery of over 90%, by employing a series of solvent extractions, adsorbent treatment, fractional precipitation and high performance liquid chromatography.

Claims

1. A method for mass production of high purity taxol from Taxus genus plant, said method comprising the steps of:

   (i) organic solvent extraction of biomass from Taxus genus plant in a mixture of dichloromethane/methanol or methanol, stirring and filtering to obtain a crude extract,

   (ii) synthetic adsorbent treatment of said crude extract at a ratio of 10 to 200% (w/w), stirring and filtering to obtain a filtrate,

   (iii) addition of 500 to 1500% volume of hexane to the filtrate in dichloromethane to precipitate crude taxol,

   (iv) fractional precipitation of the crude taxol in a mixture of methanol and water at a ratio of 1 to 10% (w/v) and drying the precipitate to obtain taxol powder, and

   (v) high performance liquid chromatography (HPLC) of said taxol powder.

2. The method according to claim 1, wherein the biomass from Taxus genus plant is the chopped and powdered leaf or bark, or the cake of cell mass obtained in tissue culture of Taxus genus plant.

3. The method according to claim 1 or 2, wherein the Taxus genus plant is selected from the group consisting of Taxus brevifolia, Taxus canadensis, Taxus cuspidata, Taxus baccata, Taxus globosa, Taxus floridana, Taxus wallichiana, Taxus media and Taxus chinensis.

4. The method according to claim 1, wherein the solvent extraction is carried out by: the addition of biomass of Taxus genus plant to methanol at a ratio of 20 to 200% (w/v), stirring at room temperature and filtering to obtain a methanol extract; and, the addition of dichloromethane to the methanol extract at a volume ratio of 10 to 50%, stirring and leaving to stand to obtain a crude extract.

5. The method according to claim 1, wherein the solvent extraction is carried out by: the addition of biomass of Taxus genus plant to a mixture of dichloromethane/methanol at a ratio of 10 to 100% (w/v), stirring at room temperature and filtering to obtain a dichloromethane/methanol extract; the dissolution of said extract in methanol at a ratio of 50 to 200% (w/v) to obtain a methanol extract; and, the addition of said extract to hexane at a volume ratio of 5 to 30%, stirring and leaving to stand to obtain a crude extract by the removal of hexane layer.

6. The method according to claim 5, wherein dichloromethane and methanol are mixed at a volume ratio of 7:3 to 9:1.

7. The method according to claim 1, wherein the synthetic adsorbent is selected from the group consisting of active clay, activated charcoal and activated carbon.

8. The method according to claim 1, wherein methanol and water are mixed at a volume ratio of 2:1 to 1:1.

9. The method according to claim 1, wherein the HPLC is composed of an HPLC employing a hydrophobic resin column and an HPLC employing a silica column.

10. The method according to claim 9, wherein the HPLC employing a hydrophobic resin column is carried out by the injection of taxol powder dissolved in organic solvent at a ratio of 0.5 to 10% (w/v) to ODS (octadecylsilylated, C_{18}) column.

11. The method according to claim 10, wherein the organic solvent is dimethylsulfoxide or methanol.

12. The method according to claim 10, wherein the elution is made with a mixture of methanol and water mixed at a volume ratio of 1:0.3 to 1:0.8.

13. The method according to claim 9, wherein the elution in the HPLC employing a silica column is made with a mixture of dichloromethane and methanol mixed at a volume ratio of 95:5 to 99:1.

Patentansprüche

1. Verfahren zur Massenherstellung von hochreinem Taxol aus Pflanzen der Gattung Taxus, wobei das Verfahren die Schritte umfaßt:

   (i) Extraktion mit organischem Lösungsmittel einer Biomasse aus Pflanzen der Gattung Taxus in einer Mischung aus Dichlormethan/Methanol oder Methanol, Rühren und Filtrieren, um einen Rohextrakt zu erhalten,

   (ii) Behandlung des Rohextrakts mit synthetischem Adsoptionsmittel in einem Verhältnis von 10 bis 200% (w/w), Rühren und Filtrieren, um ein Filtrat zu erhalten,

   (iii) Zusetzen von 500 bis 1500 Vol.% Hexan zu dem Filtrat in Dichlormethan, um Rohtaxol auszufällen,

   (iv) fraktionierte Ausfällung des Rohtaxols in einer Mischung aus Methanol und Wasser in einem Verhältnis von 1 bis 10% (w/v) und Trocknen der Ausfällung, um Taxolpulver zu erhalten, und

   (v) Hochleistungs-Flüssigchromatographie (HPLC) des Taxolpulvers.

2. Verfahren nach Anspruch 1, wobei es sich bei der Biomasse aus Pflanzen der Gattung Taxus um gehacktes und pulverisiertes Blatt oder Rinde oder den Kuchen von Zellmasse handelt, wie erhalten bei der Gewebekultur von Pflanzen der Gattung Taxus.

3. Verfahren nach Anspruch 1 oder 2, wobei die Pflanze der Gattung Taxus aus der Gruppe gewählt ist, bestehend aus Taxus brevifolia, Taxus canadensis, Taxus cuspidata, Taxus baccata, Taxus globosa, Taxus floridana, Taxus wallichiana, Taxus media und Taxus chinensis.

4. Verfahren nach Anspruch 1, wobei die Lösungsmittelextraktion ausgeführt wird durch: Zugabe von Biomasse von Pflanzen der Gattung Taxus zu Methanol in einem Verhältnis von 20 bis 200% (w/v), Rühren bei Raumtemperatur und Filtrieren, um einen Methanolextrakt zu erhalten; und durch die Zugabe von Dichlormethan zu dem Methanolextrakt bei einem Volumenverhältnis von 10 bis 50%, Rühren und Stehenlassen, um einen Rohextrakt zu erhalten.

5. Verfahren nach Anspruch 1, wobei die Lösungsmittelextraktion ausgeführt wird durch: Zugabe von Biomasse von Pflanzen der Gattung Taxus zu einer Mischung aus Dichlormethan/Methanol in einem Verhältnis von 10 bis 100% (w/v), Rühren bei Raumtemperatur und Filtrieren, um einen Dichlormethan/Methanol-Extrakt zu erhalten; die Auflösung des Extrakts in Methanol in einem Verhältnis von 50 bis 200% (w/v), um einen Methanolextrakt zu erhalten; und durch die Zugabe des Extrakts zu Hexan bei einem Volumenverhältnis von 5 bis 30%, Rühren und Stehenlassen, um durch die Entfernung der Hexanschicht einen Rohextrakt zu erhalten.

6. Verfahren nach Anspruch 5, wobei Dichlormethan und Methanol bei einem Volumenverhältnis von 7:3 bis 9:1 vermischt werden.

7. Verfahren nach Anspruch 1, wobei das synthetische Adsorptionsmittel aus der Gruppe gewählt ist, bestehend aus Aktivton, Aktivholzkohle und Aktivkohle.

8. Verfahren nach Anspruch 1, wobei Methanol und Wasser bei einem Volumenverhältnis von 2:1 bis 1:1 vermischt werden.

9. Verfahren nach Anspruch 1, wobei die HPLC aufgebaut ist aus einer HPLC unter Verwendung einer hydrophoben Harzsäule und einer HPLC unter Verwendung einer Silicasäule.

10. Verfahren nach Anspruch 9, wobei die HPLC unter Verwendung einer hydrophoben Harzsäule durchgeführt wird durch Injizieren von Taxolpulver, gelöst in organischem Lösungsmittel bei einem Verhältnis von 0,5 bis 10% (w/v) zu ODS(octadecylsylierte, C_{18})-Säule.

11. Verfahren nach Anspruch 10, wobei das organische Lösungsmittel Dimethylsulfoxid oder Methanol ist.

12. Verfahren nach Anspruch 10, wobei die Elution mit einer Mischung aus Methanol und Wasser, gemischt bei einem

dans une colonne ODS (octadécylesililée, C_{18}).

11. Procédé selon la revendication 10, **caractérisé en ce que** le solvant organique est le diméthylsulfoxide ou le méthanol.

12. Procédé selon la revendication 10, **caractérisé en ce que** l'élution est réalisée avec un mélange de méthanol et d'eau mélangés avec un rapport de volume de 1:0,3 à 1:0,8.

13. Procédé selon la revendication 9, **caractérisé en ce que** l'élution dans l'HPLC utilisant une colonne de silice est réalisée avec un mélange de dichlorométhane et de méthanol mélangés avec un rapport de volume de 95:5 à 99:1.

Volumenverhältnis von 1:0,3 bis 1:0,8, durchgeführt wird.

13. Verfahren nach Anspruch 9, wobei die Elution bei der HPLC unter Verwendung einer Silicasäule durchgeführt wird mit einer Mischung aus Dichlormethan und Methanol, gemischt bei einem Volumenverhältnis von 95:5 bis 99:1.

Revendications

1. Procédé de production en masse de taxol de haute pureté à partir d'une plante du genre Taxus, ledit procédé comportant les étapes consistant à :

   (i) Extraire avec un solvant organique une biomasse constituée d'une plante du genre Taxus dans un mélange de dichlorométhane/méthanol ou de méthanol, agiter et filtrer pour obtenir un extrait brut,

   (ii) Traiter ledit extrait brut avec un adsorbant synthétique dans un rapport de 10 à 200% (p/p), agiter et filtrer pour obtenir un filtrat,

   (iii) Ajouter au filtrat dans du dichlorométhane de 500 à 1500% en volume d'hexane pour précipiter le taxol brut,

   (iv) Précipiter de manière fractionnée le taxol brut dans un mélange de méthanol et d'eau dans un rapport de 1 à 10% (p/v) et sécher le précipité pour obtenir une poudre de taxol, et

   (v) Réaliser une chromatographie à hautes performances (HPLC) de ladite poudre de taxol.

2. Procédé selon la revendication 1, **caractérisé en ce que** la biomasse à partir d'une plante du genre Taxus est de l'écorce ou de la feuille de plante du genre Taxus coupée en morceaux et mise en poudre, ou le gâteau de masse de cellules obtenues par une culture de tissu de plante du genre Taxus.

3. Procédé selon la revendication 1 ou 2, **caractérisé en ce que** la plante du genre Taxus est choisie parmi le groupe constitué de Taxus brevifolia, Taxus canadensis, Taxus cuspidata, Taxus baccata, Taxus globosa, Taxus floridana, Taxus wallichiana, Taxus media et Taxus chinensis.

4. Procédé selon la revendication 1, **caractérisé en ce que** l'extraction par un solvant est menée en : ajoutant la biomasse de plante du genre Taxus à du méthanol dans un rapport de 20 à 200% (p/v), en agitant à température ambiante et en filtrant pour obtenir un extrait au méthanol; et en ajoutant du dichlorométhane à l'extrait au méthanol avec un rapport de volume de 10 à 50%, en agitant et en laissant reposer pour obtenir un extrait brut.

5. Procédé selon la revendication 1, **caractérisé en ce que** l'extraction par un solvant est menée en : ajoutant la biomasse de plante du genre Taxus à un mélange de dichlorométhane/méthanol dans un rapport de 10 à 100% (p/v), en agitant à température ambiante et en filtrant pour obtenir un extrait au dichlorométhane/méthanol; en dissolvant ledit extrait dans du méthanol dans un rapport de 50 à 200% (p/v) pour obtenir un extrait au méthanol; et, en ajoutant ledit extrait à de l'hexane dans un rapport de volume de 5 à 30 %, en agitant et en laissant reposer pour obtenir un extrait brut en enlevant la phase d'hexane.

6. Procédé selon la revendication 5, **caractérisé en ce que** le dichlorométhane et le méthanol sont mélangés avec un rapport de volume de 7:3 à 9:1.

7. Procédé selon la revendication 1, **caractérisé en ce que** l'adsorbant synthétique est choisi dans le groupe constitué d'argile actif, de charbon actif et de carbone activé.

8. Procédé selon la revendication 1, **caractérisé en ce que** le méthanol et l'eau sont mélangés dans un rapport de volume de 2:1 à 1:1.

9. Procédé selon la revendication 1, **caractérisé en ce que** l'HPLC est composée d'une HPLC utilisant une colonne de résine hydrophobe et une HPLC utilisant une colonne de silice.

10. Procédé selon la revendication 9, **caractérisé en ce que** l'HPLC utilisant une colonne de résine hydophobe est menée en injectant de la poudre de taxol dissoute dans un solvant organique avec un rapport de 0,5 à 10% (p/v)